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16. ABSTRACT

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Measurement of Cardiopulmonary Function by Rebreathing Methodology in Piglets

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ABSTRACT. The use of a multiple gas rebreathing method for the measurement of cardiopulmonary function in mechanically ventilated neonates was evaluated. The following indices of cardiopulmonary function were assessed in 20 piglets (mean weight, 2.3 kg): 1) pulmonary capillary blood flow (Q_c), 2) diffusing capacity for carbon monoxide (D_LCO), 3) lung gas volume (FRC), 4) oxygen consumption (V_{O_2}), and 5) volume of the pulmonary tissues and capillaries (VTPC), the latter an estimate of total lung water. During mechanical ventilation at zero end expiratory pressure, all rebreathing parameters correlated well with body weight. Additionally, a good correlation ($r = 0.81$, slope = 0.99) between VTPC and postmortem estimate of total lung water was observed. The effect of ventilation with positive end expiratory pressure (PEEP) was then studied in 10 piglets. On increasing PEEP from zero to 15 cm H_2O, FRC significantly increased by 208%, Q_c significantly decreased by 60%, and no changes in VTPC occurred. Seven piglets were then studied after induction of lung injury by oleic acid infusion. Compared with the pre-oleic acid infusion values, all the rebreathing variables decreased during ventilation without PEEP. Unlike the situation with the normal piglets, when PEEP was increased from zero to 10 cm H_2O in the oleic acid-infused piglets, the values for FRC and VTPC significantly increased. Mean VTPC at 10 cm H_2O was 20 ± 2 ml/kg which correlated well ($r = 0.93$) with the postmortem total lung water value of 19 ± 1 g/kg. Thus, multiple gas rebreathing methodology is applicable to studies using small animals. The observation that the application of PEEP in lung injury increased the accuracy of the VTPC measurement suggests that PEEP improves ventilation of injured lung segments. (*Pediatr Res* 18:1167–1172, 1984)

Abbreviations

Q_c, pulmonary capillary flow
D_LCO, diffusing capacity for carbon monoxide
V_{O_2}, oxygen consumption
FRC, lung volume
VTPC, volume of pulmonary tissue and capillary blood
PEEP, positive end expiratory pressure
BW, body weight

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The noninvasive multiple gas rebreathing method (19) allows for easily repeatable, accurate, and rapid measurement of several indices of cardiopulmonary function including Q_c, D_LCO, V_{O_2}, FRC, and VTPC, the latter measurement an accurate estimate of total lung water (10, 12). This technique has been used in large animals (dogs, sheep) as well as humans (3, 10, 11, 13, 16, 18, 19, 21). Presently, the measurement of these cardiopulmonary indices in human neonates, utilizing standard invasive techniques, is limited by the size of the vascular access, the necessity to repetitively disconnect the patient from a ventilator, and by the time required to make the measurements (20).

The purpose of the present study was to evaluate the feasibility of applying rebreathing methodology to piglets, a small animal similar in size to a human neonate, and with collateral ventilation similar to humans (8). Since PEEP is a commonly used treatment modality in neonatal lung injury, we also evaluated the effects of PEEP on rebreathing parameters in normal piglets and also in piglets with oleic acid-induced lung injury.

MATERIALS AND METHODS

Twenty newborn piglets, ages 2–20 days, with a mean body weight of 2.3 kg (range, 1.4–4.8 kg) were anesthetized with intravenous chloralose (40 mg/kg), urethane (250 mg/kg), and intramuscular ketamine (10 mg/kg). Catheters were placed in a jugular vein and femoral artery. Systemic arterial pressure was monitored using a transducer referred to the midchest level (model 1280C, Hewlett-Packard, Waltham, MA). The trachea was then surgically exposed and cut horizontally. A size 12 French polyvinyl tube was inserted in the distal trachea and secured with two silk ligatures. Body temperature was measured by a rectal probe and maintained at 39° C utilizing a servo-controlled heating lamp. The piglet was then paralyzed with intravenous pancuronium bromide (0.2 mg/kg) and placed on a constant volume ventilator (EDCO Scientific, Inc., Chapel Hill, NC) with a tidal volume of 30 ml/kg and the respiratory rate was adjusted to maintain a $Paco_2$ of 35–40 torr. Pao_2, $Paco_2$, and pH in arterial blood were measured using standard electrodes (Radiometer model BM SBMK2, Copenhagen, Denmark). In several animals, including normal piglets and piglets with lung injury, airway pressure was monitored throughout the experiment.

The rebreathing apparatus consisted of a 150-ml rubber bag-in-bottle and valve developed by Gallioto *et al.* (13) with a total dead space of 2 ml. The valve was interposed between the piglet's airway opening and the ventilator. By turning the valve, the inspiratory gas flow from the ventilator could be rapidly switched to the bag-in-bottle system allowing the animal to be rebreathed with the test gas mixture contained in the bag. The test gas mixture contained 0.3% $C^{18}O$, 0.8% C_2H_2, 10% He, 21% O_2, and the balance N_2. Rebreathing was initiated at the end of expiration and continued at a rate of 30 breaths/min for 20 s. The gases were continuously sampled at the airway opening by a mass spectrometer with a sampling rate of 15 ml/min (MGA 1100, Perkin-Elmer Corp, Pomona, CA). The analog signals from the mass spectrometer were directed to a small computer (MINC-11, Digital Equipment Corp., Maynard, MA). The computer digitized the analog signals for the gases at a rate of 15 points/s correcting each value of C_2H_2 and $C^{18}O$ for helium dilution. The program calculated the slope of the disappearance curves of $C^{18}O$ and C_2H_2 after the point of minimal oscillation of the helium tracing (19). Data was analyzed for three consecutive breaths after this starting point using the algorithm of Sackner *et al.* (19). All rebreathing runs were done in triplicate and averaged (10). No correction was made for gas back pressure, which was always <5% of the inspired gas concentrations (10, 12).

In preliminary experiments, we found a rebreathing volume of 60 ml for piglets weighing 0–2 kg, 80 ml for 2–4-kg piglets, and 100 ml for 4–6-kg piglets to be optimal in regard to reproducibility and accuracy of measurements. This is similar to rebreathing volumes per body weight used by us and others in dogs and humans (10, 11, 16, 19). Thirteen piglets were studied at zero end expiratory pressure. Ten of these animals were then ventilated with varying levels of PEEP (5, 10, 15 cm H_2O) and repeat rebreathing measurements were made while on PEEP (normal lung group). The various levels of PEEP were applied in differing sequences, and continued for a 10- to 15-min period before any measurements were performed. PEEP was applied by immersing the expiratory limb of the ventilator under water. Since the rebreathing measurement begins at end expiration in a closed system, the rebreathing method would not increase end expiratory pressure. Seven animals were again ventilated at zero end expiratory pressure and had repeat baseline rebreathing measurements performed. These seven piglets were then given an infusion of 0.05 ml/kg oleic acid via the jugular vein (lung injury group). Repeat rebreathing measurements were made 15 min after the oleic acid injection during ventilation with zero end expiratory pressure and then with the application of various levels of PEEP. Again, the various levels of PEEP were administered in randomly differing sequences as described previously in order to minimize the effect of increasing lung water during the measurement period as a result of the injury. At the conclusion of the last rebreathing measurement, the piglet was killed with an intravenous injection of KCl. The chest was rapidly opened and the lungs removed. All blood was allowed to drain passively from the lungs and then weighed (total wet weight). The lungs were then placed in an 80° C oven and dried until no further change in weight occurred (dry weight). Wet minus dry lung weight was then calculated and compared to VTPC. The justification for this comparison has been discussed in a previous publication (10).

Statistical analysis to compare the different levels of PEEP with baseline values was performed by using Dunnet's least squares difference for multiple comparisons (7). Comparison between PEEP levels on rebreathing parameters was tested by the Scheffe test (24). Linear regressions were calculated using standard algorithms. All data are expressed as the mean ± the standard error of the mean. p values <0.05 were considered to be significant.

RESULTS

Figure 1 shows the data obtained for the various rebreathing parameters *versus* body weight in all 20 animals at zero end expiratory pressure. The linear regressions of the various rebreathing parameters with body weight are shown. The average coefficient of variation for each rebreathing parameter was D_LCO (8%), VTPC (11%), $\dot{Q}_c$ (8%), FRC (5%), and $\dot{V}O_2$ (5%), demonstrating good reproducibility of the method.

Normal lung group. Table 1 lists the results obtained in the piglets for D_LCO, VTPC, $\dot{Q}_c$, FRC, and $\dot{V}O_2$ at the various levels

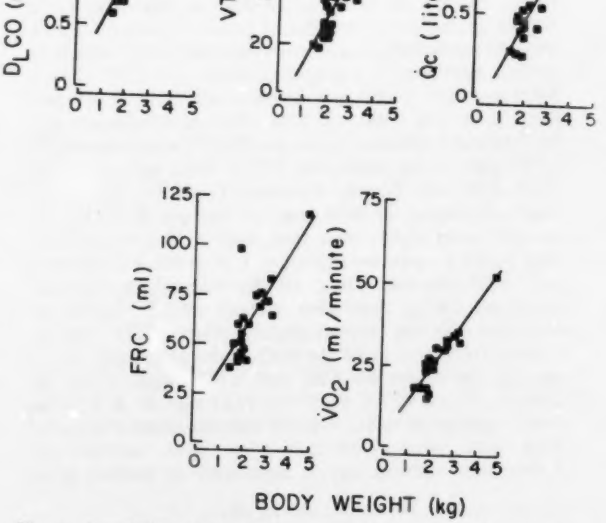

Fig. 1. Correlation of the various rebreathing parameters with BW. The regression equations and correlation coefficients (r) are: $\dot{Q}_c = -0.07 + 0.27 \times BW$ ($r = 0.80$), $D_LCO = 0.15 + 0.39 \times BW$ ($r = 0.94$), $FRC = 17.63 + 19.49 \times BW$ ($r = 0.76$), $\dot{V}O_2 = 3.30 + 10.16 \times BW$, and $VTPC = -6.13 + 18.60 \times BW$ ($r = 0.90$).

of PEEP. Increasing the level of PEEP, from 0 to 15 cm H_2O, resulted in a progressive significant increase in FRC and a decrease in Q_t. No significant changes in VTPC occurred at any level of PEEP compared to the zero PEEP baseline value. The absolute magnitude of D_LCO increased significantly at the 15 cm H_2O PEEP level as compared to the zero PEEP value. D_LCO when computed per unit lung volume decreased with increasing levels of PEEP. $\dot{V}O_2$ fell significantly by 25% only at the 15 cm H_2O PEEP level. Only four piglets could be successfully ventilated at a level of 15 cm H_2O PEEP long enough to obtain rebreathing measurements. The remainder of the animals developed pneumothoraces at this high level of PEEP as determined by chest wall transillumination and marked decreases in FRC.

The accuracy of the rebreathing VTPC measurement was determined by comparison with the postmortem wet minus dry lung weights. Figure 2 shows the linear regression for VTPC with wet minus dry lung weight in the normal piglets ($r = 0.80$; $p < 0.01$). Total wet minus dry weight averaged 79.2 ± 1.2% of total wet weight. Mean wet minus dry lung weight/kg body weight was 12.66 ± 0.60. Rebreathing measurements of $\dot{Q}_c$ were also compared to simultaneously obtained cardiac outputs using a standard indicator dilution technique following injection of indocyanine green ($\dot{Q}_T$) during rebreathing. In two piglets, mean $\dot{Q}_c$ of six determinations was 0.59 ± 0.03 liter/min as determined by the rebreathing technique and was 0.67 ± 0.05 liter/min as determined by dye dilution. The linear regression of $\dot{Q}_c$ *versus* $\dot{Q}_T$ was $\dot{Q}_c = 0.19 + 0.60 \times \dot{Q}_T$ ($r = 0.98$).

Lung injury group. Table 2 lists the rebreathing values obtained in four piglets ventilated at zero end expiratory pressure following oleic acid infusion. Paired comparison of these values with those of the baseline pre-oleic acid values (data not shown) revealed significant parallel decreases in D_LCO (14 ± 4%), VTPC (14 ±

Table 1. *Effect of PEEP on normal lungs**

PEEP (cm H_2O)	n	D_LCO (ml/min/mm Hg/kg)	VTPC (ml/kg)	$\dot{Q}_c$ (liter/min/kg)	FRC (ml/kg)	$\dot{V}O_2$ (ml/min/kg)
0	10	0.44 ± 0.02	16 ± 1	0.25 ± 0.02	26 ± 1	12 ± 1
5	8	0.48 ± 0.03	15 ± 1	0.17 ± 0.01†	44 ± 3†	11 ± 1
10	8	0.49 ± 0.02	15 ± 1	0.14 ± 0.02†	63 ± 2†	11 ± 1
15	4	0.55 ± 0.07†	17 ± 3	0.10 ± 0.01†	80 ± 6†	9 ± 0†

* Data shown as $\bar{x}$ ± SEM.
† Significant change ($p < 0.05$) from the values obtained at zero end expiratory pressure.

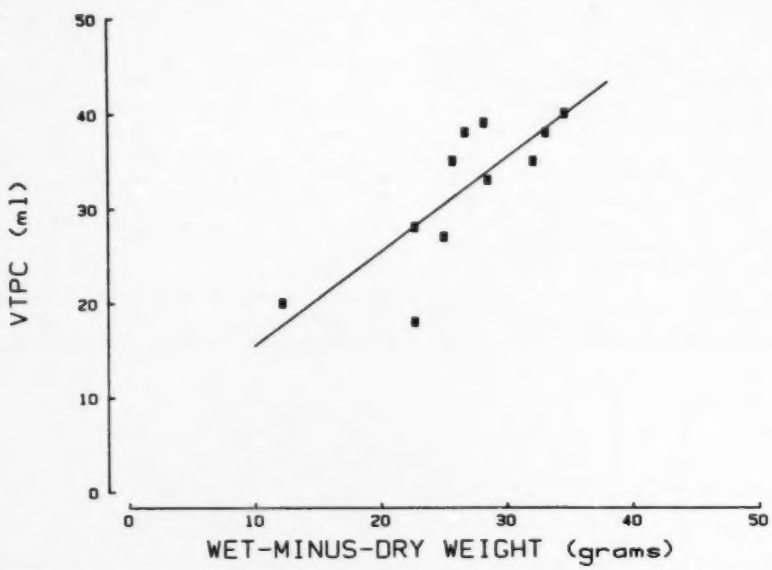

Fig. 2. Graphic representation of the relationship between VTPC and wet minus dry weight in the 11 normal piglets. VTPC was measured during ventilation at zero end expiratory pressure ($r = 0.81$; slope = 0.99).

Table 2. *Effect of PEEP on edematous lungs**

PEEP (cm H_2O)	n	D_LCO (ml/min/mm Hg/kg)	VTPC (ml/kg)	$\dot{Q}_c$ (liter/min/kg)	FRC (ml/kg)	$\dot{V}O_2$ (ml/min/kg)
0	4	0.38 ± 0.04	14 ± 4	0.19 ± 0.03	22 ± 2	12 ± 1
5	7	0.44 ± 0.05	15 ± 1	0.14 ± 0.02	37 ± 2†	10 ± 1
10	7	0.48 ± 0.03	19 ± 1†	0.11 ± 0.03†	61 ± 2†	9 ± 1
15	4	0.43 ± 0.05	22 ± 2†‡	0.08 ± 0.03†	66 ± 7†‡	6 ± 2†

* Data shown as $\bar{x}$ ± SEM.
† Significant change ($p < 0.05$) from the values obtained at zero end expiratory pressure.
‡ Significantly different from the 0 and 5 cm H_2O PEEP level but not from the 10 cm H_2O PEEP level.

6%), $\dot{Q}_t$ (20 ± 3%), and FRC (15 ± 5%) with no change in $\dot{V}_{O_2}$. The VTPC value determined at zero end expiratory pressure following the oleic acid infusion significantly underestimated the final postmortem wet minus dry lung weight in these four animals by an average of 32 ± 7%. The three other piglets in this lung injury group developed pneumothoraces when on 15 cm H_2O PEEP, necessitating discontinuance of the study prior to obtaining the zero PEEP rebreathing parameters.

Table 2 also shows the values of the rebreathing parameters obtained in all seven piglets studied while at varying levels of PEEP following oleic acid infusion. There was no significant change in arterial blood gases or arterial pressure during the experiment following oleic acid injection except for a significant increase in P_{O_2} (mean increase of 32%) when PEEP was increased from zero to 5 cm H_2O PEEP. No significant changes in arterial blood gases or systemic blood pressure at other levels of PEEP were noted (data not shown). FRC significantly increased 68% on increasing PEEP from zero to 5 cm H_2O and an additional 40% when PEEP was increased from 5 to 10 cm H_2O. No significant change in FRC occurred when PEEP was increased from 10 to 15 cm H_2O. $\dot{Q}_t$ fell at each higher level of PEEP including the change from 10 to 15 cm H_2O PEEP. The changes observed in D_LCO with the different PEEP levels were not significantly different from the control value. As in the noninjured group, a significant decrease in $\dot{V}_{O_2}$ (50%) was noted only at the 15 cm H_2O PEEP level. Following oleic acid injury VTPC did not change significantly on changing PEEP from zero to 5 cm H_2O. However, in contrast to the effects of PEEP on normal lungs, a significant increase in VTPC at the 10 cm H_2O PEEP level was observed compared to the value obtained at zero PEEP. Similar to the observed effects of PEEP on FRC with this group, the change in VTPC on changing PEEP from 10 to 15 cm H_2O was not significant. Since the rebreathing determination of VTPC was obtained in all seven animals in this group at 10 cm H_2O PEEP, and since no further significant change in VTPC occurred when PEEP was increased from the 10 to 15 cm H_2O PEEP level, we chose the VTPC value calculated at 10 cm H_2O PEEP for the comparison with postmortem wet minus dry lung weight (Fig. 3). The relationship of VTPC and wet minus dry lung weight for these animals is clearly linear ($r = 0.93$; $p < 0.01$; slope = 1.0). The correlation coefficients for VTPC and wet minus dry lung weights was 0.58 (p = NS), 0.57 (p = NS), and 0.98 ($p < 0.001$) for the 0, 5, and 15 cm H_2O PEEP levels, respectively. The VTPC determinations made at 10 cm H_2O PEEP were 98 ± 7% of the postmortem wet minus dry lung weights and had increased by an average of 35% over pre-oleic acid values. Total wet minus dry lung weight in this group was 83 ± 1% of the total wet weight. Wet minus dry lung weight/kg body weight was 19.7 ± 1.7.

DISCUSSION

We have successfully applied rebreathing methodology to measure indices of cardiopulmonary function in normal newborn piglets and also during oleic acid-induced pulmonary edema. This technique allows for on-line, rapid, safe, and easily repeatable assessment of cardiopulmonary function.

Takezawa *et al.* (20) used a modified single-breath method to measure D_LCO in rabbits (1.3–3.5 kg) which are comparable in weight to the piglets in this study. D_LCO by their method was 0.52 ml/min/mm Hg/kg. Watanabe and Frank (23), using a single-breath D_LCO method in cats similar in size to the piglets in this study, found the D_LCO to be 0.41 ml/min/mm Hg/kg. Both of these reported values are comparable to the 0.44 ml/min/mm Hg/kg value that we obtained in piglets. In our experiments, the first determination of D_LCO was not significantly different from any of the subsequent measurements made (at least two) in the normal lung, no PEEP group. Similar to previous reports, this suggests that there was no significant accumulation of $C^{18}O$ in the blood leading to an underestimation of D_LCO (10, 11, 19). Watanabe and Frank (23) also measured FRC by neon dilution in cats and obtained a value of 26 ml/kg which also compares favorably with the 30 ml/kg we found in piglets. When measured by rebreathing at zero end expiratory pressure, VTPC correlated well with the postmortem estimate of total lung water in the normal piglets (Fig. 2). This finding is similar to data reported previously from our laboratory in normal adult dogs (10, 12). The effects of PEEP on normal lungs was predictable and similar to that found in larger animals, *i.e.* an increase in lung volume and decrease in pulmonary capillary blood flow (9, 12). D_LCO computed per unit lung volume decreased with increasing levels of PEEP suggesting creation of lung zones in which alveolar pressure exceeded arterial pressure, as would be expected with the application of PEEP. The decrement in pulmonary capillary blood flow with PEEP is similar to that reported by us and others (3, 4, 9, 12) and the decrement in $\dot{Q}_t$ with oleic acid infusion and PEEP is similar to previously reported values from our laboratory in dogs (9). These data suggest that the rebreathing volumes used and airway pressures developed did

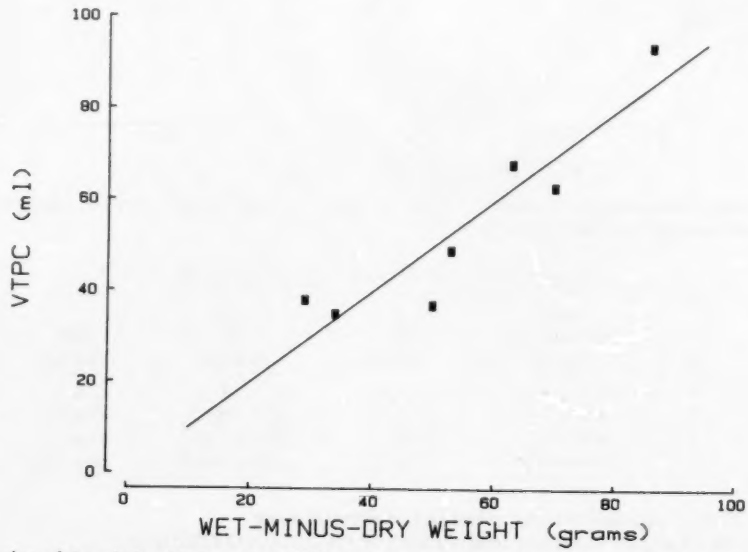

Fig. 3. Graphic representation of the relationship between VTPC and wet minus dry weight in the seven piglets following oleic acid lung injury. VTPC was measured during ventilation at 10 cm H_2O PEEP ($r = 0.93$).

the rebreathing maneuver in the present study were not excessive. Although we did not measure airway pressure directly in every experiment, when it was measured it did not exceed the pressures measured prior to or following the rebreathing maneuver.

The rebreathing measurement of VTPC, $\dot{Q}_c$, FRC, and D_LCO at zero end expiratory pressure all decreased by an average of 16% following oleic acid infusion as compared to the pre-oleic acid infusion values. The rebreathing technique is dependent upon the ability of the test gas to reach gas exchange areas of the lung and is not perfusion dependent to any great degree (17). In this acute lung injury model, whose collateral ventilation is similar to humans (8), our findings of decreased FRC and underestimation of VTPC at zero end expiratory pressure after induction of lung edema suggests loss of gas exchange units secondary to the oleic acid-induced injury, with inability of the test gas to reach these involved units. Dogs, in which most of the previous studies of the applicability of rebreathing methodology in pulmonary edema have been performed (9, 10, 16), have a much more developed degree of collateral ventilation. This possibly explains the better correlation of VTPC with gravimetric lung water without the application of PEEP in dogs with lung injury (10, 16). The application of PEEP (up to 10 cm H_2O) following oleic acid lung injury resulted in a significant increase of FRC indicating lung expansion. In the normal piglets, application of PEEP did not significantly affect the VTPC measurement. In contrast, VTPC significantly increased on changing the end expiratory pressure from zero to 10 cm H_2O in the oleic acid-treated piglets. The VTPC measurement at 10 cm H_2O PEEP correlated well with the postmortem estimate of total lung water in this group of animals with pulmonary edema (Fig. 3). We do not feel that this was simply due to an accumulation of lung water with time after oleic acid infusion since we varied the sequence of the levels of PEEP in the experimental protocol. The good correlation of VTPC with the autopsy determination of total lung water suggests that PEEP improves the accuracy of the rebreathing VTPC determinations in lung injury mainly by recruiting poorly ventilated injured gas exchange units rather than overdistending more normal gas exchange units, in which instance VTPC would not be expected to increase. This latter hypothesis is supported by the finding that the VTPC measurement was accurate at zero end expiratory pressure in the normal piglets (Fig. 2) and did not increase with the application of PEEP in these normal animals (Table 1).

The published effects of PEEP on lung water content are varied. A previous study from our laboratory demonstrated no increase in rebreathing measurements of VTPC or in gravimetric lung water content with PEEP. This conclusion is also supported by other investigators that report no change in lung water utilizing measurements of gravimetric lung water or transvascular fluid filtration rates with the application of PEEP (12, 14, 25). Others have noted an increase in the amount of lung water with PEEP under certain specific experimental conditions (1, 6, 15). Our findings regarding rebreathing measurements of VTPC in piglets in this study as well as in dogs in a previous study from our laboratory (12) does differ from the study by Cassidy *et al.* (3), who reported an increase in rebreathing VTPC in humans with the application of 10 cm H_2O PEEP. We cannot explain the difference between these studies. Our present study directly compared gravimetric lung water measurements to rebreathing measurements of VTPC, and demonstrated no increase in VTPC or in gravimetric lung water content with PEEP.

Interestingly, $\dot{V}_{O_2}$ fell significantly at the 15 cm H_2O PEEP level in both the normal and lung injury groups. Tucker and Murray (22) have shown that PEEP induces changes in distribution of peripheral blood flow which might then compromise oxygen delivery to some organs. Also, oxygen uptake has been shown to be reduced in patients with adult respiratory distress syndrome who are being ventilated with PEEP (5). Danek *et al.* (5) suggested that the reduction in oxygen uptake was related to decreases in peripheral blood flow, an inability to utilize oxygen at the cellular level, or a combination of both effects. It is interesting to speculate that the decreased $\dot{V}_{O_2}$ observed at 15 cm H_2O PEEP in the piglets was related to decreased oxygen delivery secondary to the observed very low $\dot{Q}_c$.

The use of this rebreathing technique thus allows for accurate measurement of cardiopulmonary indices in small animals that would otherwise have required a more invasive approach or repetitive disconnection from a ventilator. Additionally, the application of PEEP appeared to improve the accuracy of rebreathing measurements in injured lungs presumably by improving the ventilation to injured lung gas exchange units. These data also suggest that rebreathing methodology can be used to noninvasively estimate cardiopulmonary function in humans of similar size, *i.e.* neonates, and possibly aid in determining the levels of PEEP which result in optimal gas exchange in these infants.

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REFERENCES

1. Albert RK, Lakshminaryan S, Kirk W, Butler J 1980 Lung inflation can cause pulmonary edema in zone 1 of in situ dogs. J Appl Physiol Respir Environ Exercise Physiol 49:815
2. Cander L, Forster RF 1959 Determination of pulmonary parenchymal tissue volume and pulmonary capillary blood flow in man. J Appl Physiol 14:541
3. Cassidy SS, Eschenbacher WI, Robertson CH, Nixon JV, Blomquist G, Johnson RL 1979 Cardiovascular effects of positive-pressure ventilation in normal subjects. J Appl Physiol Respir Environ Exercise Physiol 47:453
4. Cassidy SS, Robertson CH Jr, Pierce AK, Johnson RL Jr 1978 Cardiovascular effects of positive end-expiratory pressure in dogs. J Appl Physiol Respir Environ Exercise Physiol 44:743
5. Danek SJ, Lynch JP, Weg JG, Dantzker DR 1980 The dependence of oxygen uptake on oxygen delivery in the adult respiratory distress syndrome. Am Rev Respir Dis 122:387
6. Demling RH, Staub NC, Edmonds LH Jr 1975 Effect of end-expiratory airway pressure on accumulation of extravascular lung water. J Appl Physiol 38:907
7. Dunnett CW 1955 A multivariate comparison for comparing several treatments with a control. J Am Statist Assoc 50:1096
8. Fenley DC, Picken J, Welchel L, Ruff F, Corry PM, Macklem PT 1972 Blood gas transfer after small airway obstruction in the dog and minipig. Respir Physiol 15:39
9. Friedman M, Gammon K 1981 Comparison of the effects of pre- and post-injury applications of positive end expiratory pressure (PEEP). Am Rev Respir Dis 123:256
10. Friedman M, Kaufman SH, Wilkins SA Jr 1980 Analysis of rebreathing measurements of pulmonary tissue volume in pulmonary edema. J Appl Physiol Respir Environ Exercise Physiol 48:66
11. Friedman M, Kovitz KL, Miller SD, Marks M, Sackner MA 1979 Hemodynamics in teenagers and asthmatic children during exercise. J Appl Physiol Respir Environ Exercise Physiol 46:293
12. Friedman M, Wilkins SA Jr, Rothfeld AF, Bromberg PA 1984 Effect of ventilation and perfusion imbalance on inert gas rebreathing variables. J Appl Physiol Respir Environ Exercise Physiol 56:364
13. Gallioto FM, Brudno DS, Howard RH, Rivera O, Ruttiman UE 1981 New small volume rebreathing system to measure cardiopulmonary function. Pediatr Res 15:463
14. Hopewell PC, Murray JF 1976 Effects of continuous positive-pressure ventilation in experimental pulmonary edema. J Appl Physiol 40:568
15. Johnson RL, Cassidy SS, Haynes M, Reynolds RL, Schulz W 1981 Microvascular injury distal to unilateral pulmonary artery occlusion. J Appl Physiol Respir Environ Exercise Physiol 51:845
16. Peterson BT, Petrini MF, Hyde RW, Schreiner BF 1978 Pulmonary tissue volume in dogs during pulmonary edema. J Appl Physiol Respir Environ Exercise Physiol 44:782
17. Petrini MF, Peterson BT, Hyde RW 1978 Lung tissue volume and blood flow by rebreathing: theory. J Appl Physiol Respir Environ Exercise Physiol 44:795
18. Reinhart ME, Hughes JR, Kung M, Abraham WM, Ahmed T, Eyre P, Wanner A 1979 Determination of pulmonary blood flow by the rebreathing technique in airflow obstruction. Am Rev Respir Dis 120:533
19. Sackner MA, Greenelitch W, Heiman MS, Epstein S, Atkins N 1975 Diffusing capacity, membrane diffusing capacity, capillary blood volume, pulmonary tissue volume, and cardiac output measured by a rebreathing technique. Am Rev Respir Dis 111:157
20. Takezawa J, Miller FJ, O'Neil TJ 1980 Single breath diffusing capacity and lung volumes in small laboratory animals. J Appl Physiol Respir Environ Exercise Physiol 48:1052
21. Triebwasser JH, Johnson RL Jr, Burpo RP, Campbell JC, Reardon WC, Blomquist CG 1977 Non-invasive determinations of cardiac output by a

modified acetylene breathing procedure utilizing mass spectrometer measurements. Aviation Space Environ Med 48:203
22. Tucker HJ, Murray JF 1973 Effects of end expiratory pressure on organ blood flow in normal and diseased dogs. J Appl Physiol 34:265
23. Watanabe S, Frank R 1975 Lung volumes, mechanics, and single-breath diffusing capacity in anesthetized cats. J Appl Physiol 38:1148
24. Wilcox RE, Hightower WL, Smith RV 1979 Post-hoc data analysis in biomedical research. Am Lab 11:32
25. Woolverton WC, Bryham KL, Staub NC 1978 Effect of positive pressure breathing on lung lymph flow and water content in sheep. Circ Res 42:550

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